on one female indicated that shedding the wings was an active process, occurring in this instance approximately 15 minutes after the last mating act ended. Repeated contortions, somersaulting, and wing flexing were exhibited by the female during wing shedding.

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Sex-Distinctive Chromatin and the Frequency of Males in the Moth Ear Mite

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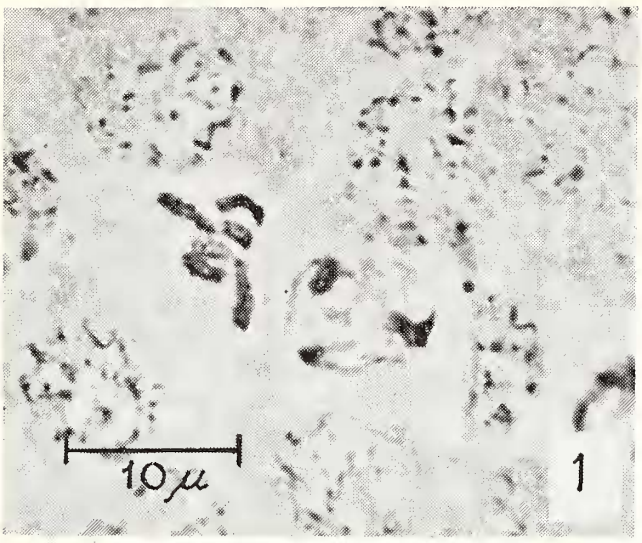
Abstract Interphase cells containing single, comma-shaped chromatin masses were seen in aceto-orcein squashes of adult males, and in a small percent of the embryos, larvae, and protonymphs of the moth ear mite, *Dicrocheles phalaenodectes*. Such cells were not found in deutonymphs or in females. When passed through a succession of hosts, fertile females produced males at intervals throughout their period of oviposition; virgin females laid only inviable eggs. Out of a total of 594 mites representing the F_1 progeny of seven females, approximately 6.8% either developed into adult males or were judged from the presence of comma cells in their early stages to be potential males. On any individual host, the first eggs usually included one or more potential males, and since the deutonymphal stage may be omitted in male ontogeny, a male was normally among the first mature mites in any colony.

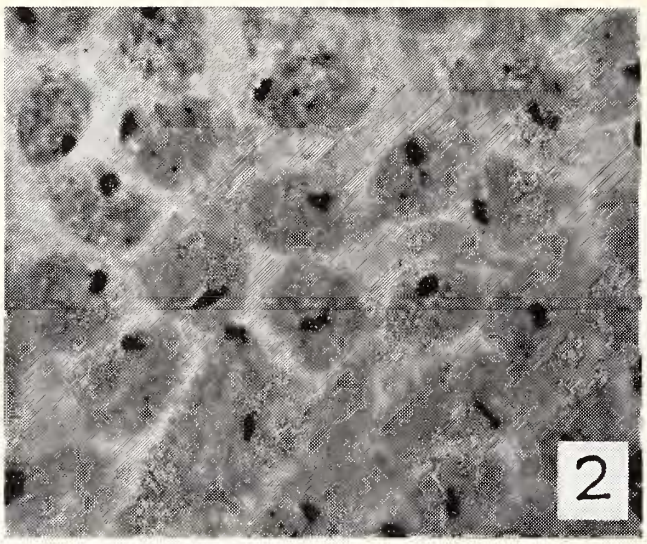
Young colonies of the moth ear mite *Dicrocheles phalaenodectes* (Treat, 1954, 1956, 1958) commonly include one or two males and many immature mites which eventually become females. Many of these females leave the host before engorgement, while some stay in the colony to become engorged and

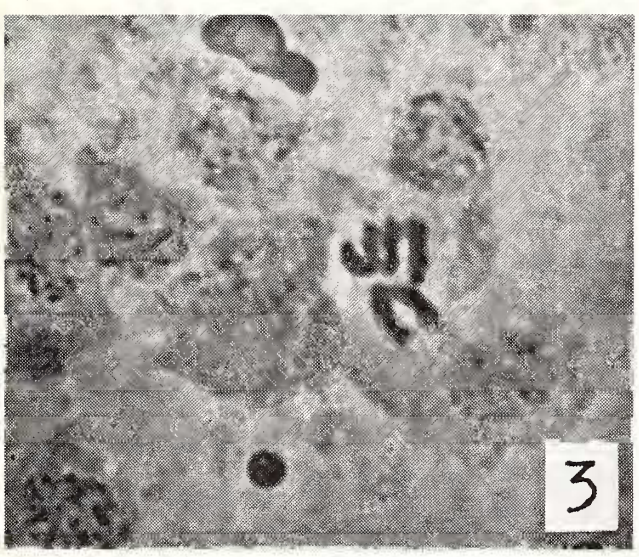
contribute to the brood. The first males develop within the tympanic air sac and cannot usually be seen unless the host is dissected, which, of course, ends the normal life of the colony. The males never leave the host. In old and once populous colonies there may be ten or more males with only a few late-developing larvae or immature females. Because of these circumstances, and because the males cannot be recognized by external features until adulthood, reliable estimates of the sex ratio are not readily obtained.

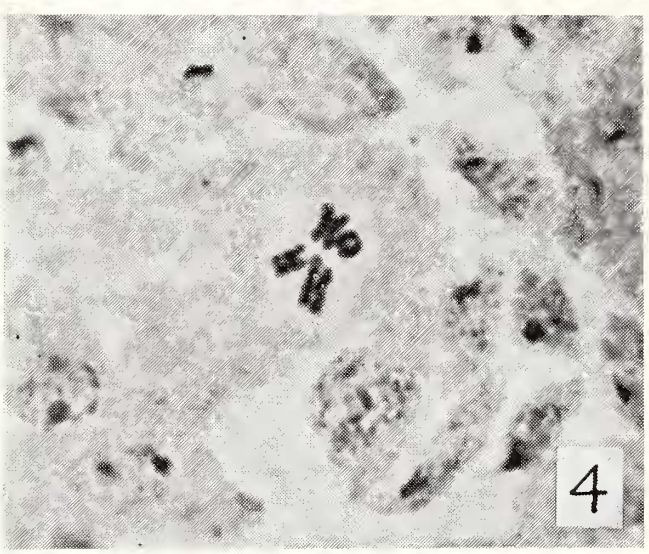
Seeking to learn something of the population structure and the mechanism of sex determination, I made aceto-orcein squashes of several hundred eggs collected from hosts in Tyringham, Massachusetts, U.S.A., during the summers of 1963 and 1964. Intrauterine and freshly laid eggs usually did not yield recognizable nuclei or mitotic figures, but after about 6 to 12 hours, when the embryo had attained some few hundred to several thousand cells, mitotic figures were often numerous and interphase nuclei abundant. In most instances the division stages showed six optically distinct chromosomal elements, and the interphase nuclei were vesicular, with the maculated appearance characteristic of relic chromosomal coils (Figs. 1, 3, 5). An occasional embryo, however, presented a different picture. In these, the metaphase plates most often showed three chromosomal elements of normal appearance, and a fourth element much shorter and denser than the others (Figs. 4, 6). In these embryos almost all of the interphase nuclei showed, in addition to fine maculations, a conspicuous comma-shaped or fusiform heterochromatic mass lying just within the nuclear membrane, or, when this was not visible, near the periphery of the cell (Fig. 2). Noting that these unusual embryos were most often from the earliest eggs laid, and that contrary to my previous impressions (Treat, 1958) these eggs were almost always placed in the tympanic air sac, which is where the first males develop, I began to suspect that the comma-containing embryos were those of males. This suspicion was strengthened by the finding of many cells with commas in all orcein squashes of adult males and in a small percent of larvae and protonymphs, especially those that developed earliest. Such cells did not appear in squashes of either deutonymphs or adult females. Especially suggestive was the discovery of commas in a chance specimen of a pharate male enclosed in an apparently protonymphal cuticle.[1] These observations make it

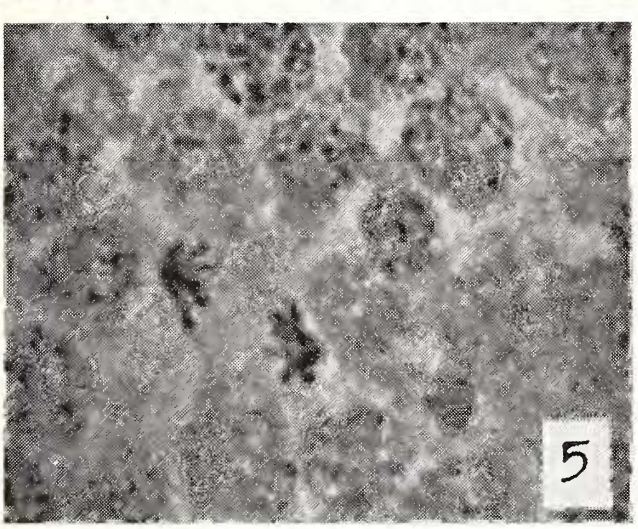
[1] A second pharate male was found later in formalin-preserved material. These specimens, together with the generally protonymphal character of the adult male chaetotaxy, suggest that the deutonymphal stage is omitted in male ontogeny. Accelerated development of early laid eggs would insure there being males available for the impregnation of the earliest maturing females. I am puzzled, however, by finding in a single host three mites with typical male chelicerae and with comma cells, but with no sign of a gonoduct or gonopore. The chaetotaxy of these specimens seems to be that of the adult male, with thickened leg setae not found in the exuvial cuticula of the pharate males. It may be that in callow specimens the gonoduct is not sufficiently sclerotized to resist flattening under the pressure applied in making a squash.

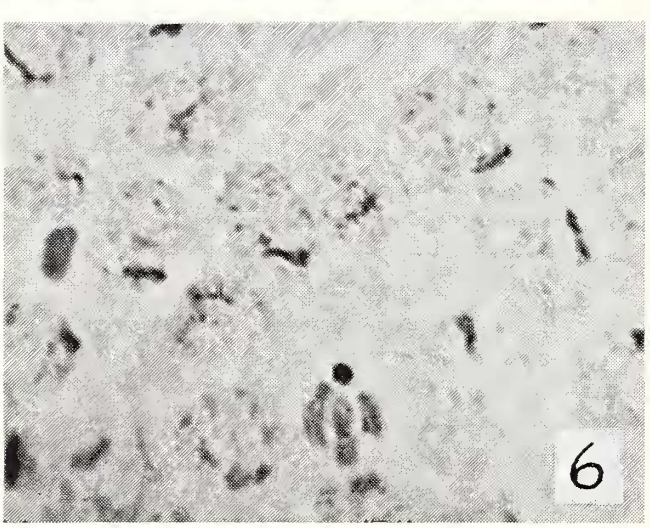




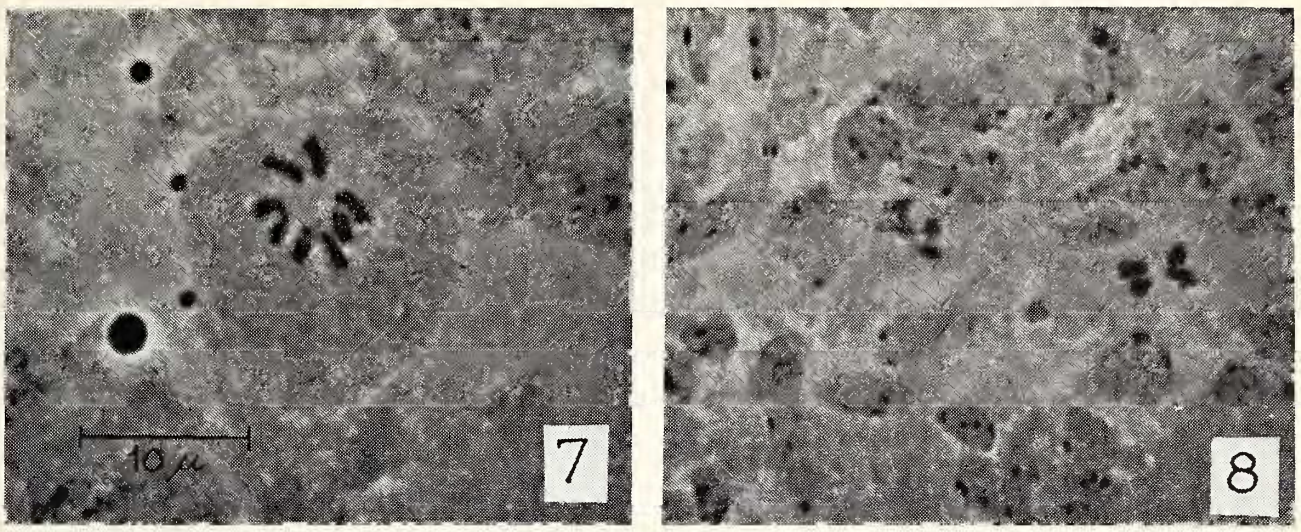








FIGS. 1–6 are dark contrast phase photographs of cells from temporary aceto-orcein squashes of *Dicrocheles phalaenodectes* embryos, taken with a 90× objective and a 15× ocular. Exposure times varied; the scale is the same for all figures. Fig. 1: interphase cells, a prophase, and a premetaphase from a typical, presumptively female embryo; the number of chromosomal elements is six. Fig. 2: interphase cells from a "male" embryo, showing comma-like chromatin masses. Fig. 3: a metaphase plate from a "female" embryo; the number of chromosomal elements is six. Fig. 4: comma cells and a metaphase plate from a "male" embryo; the number of chromosomal elements is four. Fig. 5: an anaphase from a "female" embryo. Fig. 6: a late prophase and several interphase cells from a "male" embryo; note the elongated commas resembling partly condensed chromosomes, and the pronounced heteropycnosis of the short element in the late prophase.



Figs. 7 and 8 are dark contrast phase photographs of cells from temporary aceto-orcein squashes of embryos of *Amblyseius cucumeris*. The scale is the same for both figures. Fig. 7: a metaphase plate from a typical embryo; the number of chromosomal elements is eight. Fig. 8: a metaphase plate, a prophase, and several interphase cells containing multiple chromatin masses from an atypical embryo; the number of chromosomal elements is four.

probable that from at least some early stage of embryogeny, male moth ear mites can be distinguished from females by the presence of interphase "comma cells."

In an embryo of some 20,000 cells (average interphase cell diameter about 10 μ), the commas measured about 1.2 by 2.4 μ. In many of the commas there was a minute, round, pale area in the thickest part. In addition to the comma, one to five or more small chromatic objects of granular appearance could be seen in some of the cells, and in some, a larger "granule" about a quarter to a half the size of the comma. These objects, though conceivably karyomeres such as are said to occur in the cleavage stages of *Pediculoides* and *Pediculopsis*, might well be merely portions of relic coils standing erect in the visual axis and therefore optically denser than other portions not so situated. In orcein squashes of adult males and in serial sections stained with Heidenhain's iron hematoxylin, the commas were seen in the cells of the epidermis and in the nerve cells surrounding the central ganglionic mass. They did not appear in the testes.

A direct test of the potential sex of a comma-containing egg is not at present possible, since the embryo must be killed before the commas can be seen. It would be helpful if the presence of commas could be correlated with the genetic sex as determined by chromosome structure, number, or distribution, but the material thus far examined does not provide an understanding of the chromosomal difference between male and female embryos. Figs. 1, 3, and 5 suggest a pattern of six chromosomal elements in the comma-free embryos, while Figs. 4 and 6 suggest that in the exceptional, comma-containing embryos there are but four elements, of which one is strongly heteropycnotic at late prophase. I use the term *chromosomal elements* rather than simply *chromosomes* because of

a suggestion that the sets may include one or more V-shaped chromosomes with achromatic centers, and that the apparent number of rods thus may not coincide with the actual number of chromosomes. In any event, if the commas are truly associated with maleness, as I believe, there is a striking but unexplained chromosomal difference between male and female embryos, the understanding of which will require analysis of the earliest zygotic cleavages.

Although parthenogenesis could lead to chromosomal differences (Oliver *et al.*, 1963), there is as yet no evidence of parthenogenesis in *Dicrocheles*. Out of eight females known to be virgins, four abandoned fresh experimental hosts without laying any eggs; one failed to survive the early death of its host, and three laid eggs which at first looked normal but later became discolored and collapsed without producing any larvae. Squashes of some of these eggs at ages when the embryos should have been well developed showed their contents to be apparently amorphous. It is possible, of course, that insemination might be required even for the development of haploid eggs. Striking chromosomal disparities between the sexes are evident in many animals where eliminations occur during cleavage, where there are sex-limited chromosomes, or where there are unusual phenomena of the sort discovered by the Schraders (Hughes-Schrader, 1948) and by Brown and others (Brown and Nur, 1964) in coccids. The two sectioned males of *Dicrocheles* thus far studied do not show recognizable meiotic figures, nor has meiosis been observed in the female germ line.

The origin of the commas likewise remains unexplained. They appear very clearly in 8-μ sections of embryos stained with the Feulgen reagent, and may therefore be supposed to consist of or to contain DNA. The resemblance to the sex chromatin masses of certain insects (Smith, 1945) and other animals is obvious. In early embryos containing the four-unit metaphases, many cells show what appear to be incipient commas in the form of more or less elongated, thread-like masses with varying degrees of condensation (Fig. 6). Many different interpretations might be proposed, but it seems wisest to avoid speculation until more evidence is at hand. Meanwhile, it may be of interest that heterochromatic bodies, often appearing comma-like, were seen in 4 out of 27 embryos of the common predatory phytoseiid *Amblyseius cucumeris* Oudemans (Fig. 8). In these, as in *Dicrocheles*, mitotic figures in the atypical embryos differ from those of the more usual type in having a markedly lower number of chromosomal elements (Fig. 7). There were too few mitotic figures, however, for any conclusion to be drawn, and I did not see commas in the adults of either sex.

In order to determine whether a single female moth ear mite can produce more than one male, and if so at what stages of her period of oviposition, I transferred seven mites, individually, through a succession of from three to eight hosts, keeping each host for 4 or 5 days after its mite had been removed, and then examining all of the F_1 progeny. During midsummer, 5 days are usually enough for the eggs to hatch and the nymphs to reach maturity without

danger of the colony's being augmented (and thus, in a sense, "contaminated") by a second brood of eggs. I determined the sex of adult progeny by external characters; with eggs and immature mites, I assumed that the presence of comma cells was diagnostic for maleness. Out of a total of 594 F_1 progeny, 33 were lost or accidentally destroyed before their sex could be determined. Of the remaining 561, a total of 38 or 6.8% were males. Of these, 17 were determined as adults. The largest number of males produced by a single mite was 11, the smallest number, 2. One of the mites produced males of each of six successive hosts, and all the mites but one, which was killed accidentally during its second transfer, produced males on more than one host. Males were among the latest as well as among the earliest offspring.

Regardless of the method of sex determination, these results raise interesting questions regarding the factors that govern the production of males. The transfer experiments showed that while two or more males may be produced in close succession and certainly within a day or two of one another, as much as 3 days may elapse without the production of any males. Males developed in equal numbers on hosts of both sexes. One of the transferred mites produced males on hosts of four different species, but this mite as well as several others also produced males on different individual hosts of the same species. One mite, not included among the seven previously mentioned, was induced experimentally to lay eggs successively in the right and then in the left ear of the same host. In this instance, three adult males were later found in the right ear, and two presumptively male protonymphs (i.e., with commas) in the left. Although in a freshly occupied ear, "male" eggs are commonly laid in the dorsomedial part of the tympanic air sac, it can happen that when the internal chambers of the ear become crowded, additional "male" eggs are placed in the tympanic recess. This probably explains why adult males are occasionally found in this external situation in old colonies. In comparatively rare instances, young colonies contained no males at all. Such colonies furnished the virgin females mentioned earlier. The production of males is not seasonal, at least in the northern range of the mite, for adults of both sexes were found in the usual proportion in the earliest (May) and the latest (October) colonies examined. In places where the hosts fly throughout the year, the composition of the colonies does not appear to vary significantly.

Acknowledgments Dr. James H. Oliver, Jr., of the Univ. of Cal., Berkeley, read the manuscript and compared the chromosome picture in California specimens of the moth ear mite with that in the Massachusetts material. Dr. D. A. Chant of the Univ. of Cal., Riverside, provided the species determination of *A. cucumeris*. Dr. James C. King of New York University and Dr. Louis Levine of the City University helped me in various ways. Miss Helen Ghiradella, then at Cornell University, prepared serial sections of the adult mites. To all of these people I am very grateful. I am particularly indebted to Dr. Kenneth W. Cooper of the Dartmouth Medical School, who supplied cultures of *A. cucumeris*,

sectioned and stained some of the *Dicrocheles* embryos, read the manuscript, and gave me invaluable guidance in the cytological aspects of the work.

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Peale's *Lepidoptera Americana* (1833)

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Abstract This paper locates Peale's copy of **Lepidoptera Americana** (1833) in the Library of the Amer. Mus. of Nat. Hist. and not in the British Mus. (Nat. Hist.) as claimed by Brower. The locations of eight other copies are given and Peale's copy collated in detail. Bibliographic references deal with publications and activities of Peale.

In a recent number of the *Lepidopterist's News* ("1958" [1959], pp. 101–102) Dr. Lincoln Brower published some interesting notes on Peale's **Lepidoptera Americana** with special reference to the authorship and correct name of *Papilio multicaudatus* Kirby, 1894. Unfortunately, this paper is in error concerning Peale's work and the location of the original copy.

Lepidoptera Americana, started by Titian Ramsey Peale in 1833 and apparently abandoned about 1836, is a mystery to lepidopterists. It is the first book begun by an American author on American lepidoptera that was published in this country. Peale was curator of the Philadelphia Museum and later became curator of the Academy of Natural Sciences of Philadelphia where his collection remains. Titian's father was Charles Willson Peale, who founded Peale's Museum, a private enterprise, also known as the Philadelphia Museum. A commemorative stamp in his honor, issued on January 15, 1955 by the U. S. Post Office Department, depicts the elder Peale holding a curtain open beyond which may be seen a picture gallery.